

Advances in biology through agronomy, aquaculture, coastal and environmental sciences

Leandris Argentel Martínez
Ofelda Peñuelas Rubio

Editors



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**Leandris Argentel Martínez
Ofelda Peñuelas Rubio**

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agronomy, aquaculture, coastal and
environmental sciences**



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Telefone (66) 99682-4165 (Whatsapp).
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Prologue

Advances in biology through agronomy, aquaculture, coastal and environmental sciences is an electronic book, edited by Pantanal Editora, based on the compilation of research papers where the authors of the different chapters have used highly current scientific methodologies and research equipment.

The biological sciences as the main object of research in agriculture, aquaculture, coastal and environmental sciences generate every day an understanding of knowledge that allows raising the scientific level of society as part of universal access to knowledge.

This book mainly addresses issues related to the use of plants extracts as sustainable alternatives for biocontrol of pests and bacterial diseases. It also brings together information on viruses and other diseases in aquatic organisms. In addition, studies of mangroves structure and their contribution to carbon sinks in experimental sites in northwestern Mexico are presented. Finally, an analysis on educational strategies for environmental education based on plant biology is carried out.

Editors appreciate the participation of the authors who have come from higher education institutions and research centers of great scientific prestige in Mexico. The majority of them are members of the National Research System of CONACyT, Mexico.

The authors


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Yellow head syndrome virus, a latent problematic for western aquaculture. A review


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Alba Rocio Ochoa Meza¹ 

Luz Angélica Ávila Villa^{3*} 

Roberto Rodríguez-Ramírez² 

Joe Luis Arias Moscoso¹ 

Francisco Cadena Cadena¹ 

Martina Hilda Gracia Valenzuela¹ 

ABSTRACT

In shrimp culture around the world, health is an important issue. Being the viruses the main factor in America in terms of mortalities in shrimp production. Among which the White Spot Syndrome Virus (WSSV), Taura Syndrome Virus (TSV), Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV), *Penaeus Vannamei* Nodavirus (PVNV) or even Yellow Head Virus (YHV) stand out. According to reports issued by the epizootic international organization (OIE), these are one of the main lines of research in many institutions. With YHV being one of the most important since it is one of the most aggressive pathogens in Thai aquaculture, becoming a threat to aquatic production systems worldwide. Nevertheless, advances in molecular biology, handling skills and sanitary pursuit of the units of aquatic production. Allow us to understand the strategies of replication, infection and distribution of the same unit. The purpose of this review is to summarize the progress of research on the YHV complex and its genotypes and distribution in wild and cultured shrimp around the world. In addition, different methods are used for its detection (presumptive and confirmatory analysis) and strategies against YHV.

Keywords: Shrimp, Virus, YHV.

INTRODUCTION

In aquatic activity, mainly shrimp farming, the spread of viral diseases is one of the most important problems to address since viruses represent one of the most abundant pathogens in the ocean. At present, up to 20 different attacking-peneid viruses have been identified, including White Spot

¹Tecnológico Nacional de México/Instituto Tecnológico del Valle del Yaqui. Bacum, Sonora, México.

²Laboratorio de Biotecnología y Trazabilidad Molecular de los Alimentos, Departamento de Biotecnología y Ciencias Alimentarias, Instituto Tecnológico de Sonora, 5 de Febrero 818 Sur, 85000 Ciudad Obregón, Sonora, México.

³ Departamento de Ciencias de la Salud, Universidad de Sonora, Campus Cajeme, C.P 85199, Cd. Obregón, Sonora, México

*Corresponding author: luz.avila@unison.mx

Syndrome Virus (WSSV), Taura Syndrome Virus (TSV), Infectious Hypodermal Hematopoietic Necrosis Virus (IHHNV) and Yellow Head Syndrome Virus (YHV) (Lightner et al., 2012).

Despite efforts such as biosecurity and better management practices in production systems carried out in aquaculture, the dispersion of pathogens (mainly viral) is a reality and one of the main problems in terms of health or performance reduction, since the rapid spread of pathogens is due to activities such as recreational and commercial fishing, as well as the wide range of existing hosts in these community environments. Among the pathogens of importance for shrimp culture are YHV, which has been submitted mainly in Asian countries. In 1992, it was first detected in black tiger shrimp (*Penaeus monodon*) and caused large economic losses in Thailand and Vietnam. In 1995, this virus caused losses of 5000 tons, which represents up to 40 million dollars in the Indo-Pacific region. YHV is closely related to gill-associated virus (GAV), which has caused havoc in *P. monodon* culture systems in Australia since 1996 and has been shown to be a chronic infection of wild organisms of eastern Australia.

Since 2012, YHV has been found on the sanitary code list for aquatic animals by the World Animal Health Organization (the OIE–Office International des Epizooties). Currently, YHV has been found in other Asian countries, such as Taiwan, India, China, the Philippines, Indonesia, Sri Lanka and Malaysia. In Australia, India, Indonesia, Malaysia, Mozambique, the Philippines, Taiwan, Thailand and Vietnam, GAV has been detected, and other genotypes of YHV (YHV complex) have been detected in apparently healthy *P. monodon* (Walker et al., 2001).

YHV in America is considered an exotic disease, and at present, its detection has not been confirmed by pertinent institutions. Despite this in 1995, there was one suspect of YHV in Texas (USA) in a WSSV co-infection, and later between 1995-2000, it was detected together with WSSV in frozen shrimp for importation. Subsequently, the presence of YHV was suspected in Central America between 1999-2000 and in Ecuador from 2000-2001. Nonetheless, Alday (2000) refers to the presence of YHV in a non-YHV-associated pathology, since the sequences of the amplification with five sets of primers allowed the identification of all the complexes of YHV, and only one obtained positive results for YHV.

In Mexico, there are reports of this disease in wild organisms of *Penaeus stilirostris* and cultured *Penaeus vannamei* in the country's northeast coasts, which, despite not being official reports emitted by OIE, create uncertainty in the productive sector. However, the majority of America, eastern Africa and some specific zones from eastern and southeastern Asia can be considered YHV free, according to established rules by OIE (Lightner et al., 2012).

Description of the etiologic agent of YHD

Yellow head disease (YHD) is caused by an envelope; bacilliform singled stranded positive RNA virus (40-60 nm x 150-200 nm) with a grooved nucleocapsid. This virus contains three important structural proteins: two glycoproteins of the coat, gp64 (63-67 kDa) and gp116 (110-135 kDa), and the viral nucleocapsid protein p20 (20-22 kDa) (Stentiford et al., 2009).

YHV is classified within the genus *Okavirus*, from the *Roniviridae* family, inside of the order *Nidovirales* (Liu et al., 2009); it is known that *Toro*, *Arteri* and *Coronavirus* are related (Jitrapakdee et al., 2003). RNA viruses have developed a wide range of strategies to express their genes, and a very recurrent strategy is the synthesis of subgenomic RNAs (sgmRNAs) (Miller and Koev, 2000). Nidovirals such as *Toro*, *Arteri*, *Coronavirus* and *Okavirus* differ evidently in genome size, architecture of the virion and host ranges, but their common ancestor is evident due to the identity of sequences in their replicase proteins and the similitude of the organization of their genomes, the order of the genes and the strategy of replication (Cowley et al., 2000).

In the 5' end genome of all Nidovirus, over two-thirds of the genome is used for the translation of the polyprotein replicase gene (ORF1a gene); downstream, a small gene group is found, which expresses a cluster of sgmRNAs, and all keep at the 5' end a leader sequence that is derived from 5' end genomic RNA and fused to the body of the transcript. *Okavirus* shows discontinuous transcription similar to *Torovirus*, unlike *Arteri* and *Coronavirus* (Vliet et al., 2002).

On YHV structural proteins, it has been confirmed that in virions, only the gp116 and gp64 proteins are glycosylated, unlike the p20 nucleoprotein (Soowannayan et al., 2010). Jitrapakdee et al. (2003) reported that the proteins gp116 and gp64 are encoded inside ORF 3 of the genome of YHV, which encodes the pp3 polyprotein of 1666 amino acids (aa), which contains six hydrophobic regions and undergoes posttranslational cleavage to produce a polypeptide of 228 aa of unknown function, as well as the envelope proteins gp116 and gp64 of 899 aa and 539 aa, respectively.

Afterward, Soowannayan et al. (2010) described that gp116 forms prominent projections on the coat surface of the mature virions of YHV. However, both gp116 and gp64 proteins are suspected to play a crucial role in host cell entrance. YHV infection in cells of primary cultures of lymphoid organs can be neutralized by antibodies to the gp116 glycoprotein coat but not gp64 antibodies (Assavalapsakul et al., 2005). It has been shown that a deletion of 162 nucleotides that correspond to 54 aa in the Ratchaburi/2006 chain in comparison with the Chachoengsao/1998 chain indicates a loss of six cysteine-conserved residues, which triggers a deformation in gp116, which, despite reducing the incorporation of virions inside the cell and eliminating the main sites of neutralization, keeps the virus highly infectious, virulent and able to spread (Sittidilokratna et al., 2008).

Gangnonngiw et al. (2009) reported a kind of recombinant, atypical YHV (A-YHV) that is non virulent in addition to the others already evidenced YHV-1 and YHV-2, and such virulence is more associated with the ORF1b sequence than with the ORF3 sequence despite the large deletion of the last one. The YHV ORF2 gene encodes a basic protein with 146 aa that shows a high level of identity (84.9%) with the GAV nucleoprotein and is homologous to the GAV ORF2 gene. It is clear that this gene is equivalent to the non glycosylated YHV structural protein. On the other hand, a reactive monoclonal antibody for YHV p20 has recently presented binding to the nucleocapsid of YHV virions. The finding that the ORF2 gene encodes the protein N made it possible to distinguish between *Okavirus* crustaceans

and vertebrates Nidovirus, in which the N protein gene resides in the 3' end region downstream of the genes that encode the glycoproteins and membrane proteins of the virion, while the Okavirus encoding the N protein is upstream of glycoproteins (Cowley et al., 2004). The unusual location of the gene nucleoproteins YHV and GAV could be a consequence of genetic recombination in ancestral Nidovirus, since the high frequency of genetic recombination occurs in virus (+) ssRNA, and it was also proposed that the structural diversity and Nidovirus morphology is due to modular evolution, resulting in an exchange process of complete gene recombination or gene sets (Sittidilokratna et al., 2006).

Recently, Thapanan (2014) reported that the expression of Pm clathrin AP17 in shrimp promotes the entry of virions into cells via endocytosis, increasing the speed of propagation of YHV, and a treatment based on chlorpromazine can inhibit viral replication of YHV in early stages. There are reports of proteins that act to the host benefit, supporting the dispersion of granular hemocytes, the same ones that act as the first line of defense of organisms against YHV (Havanapan, 2016).

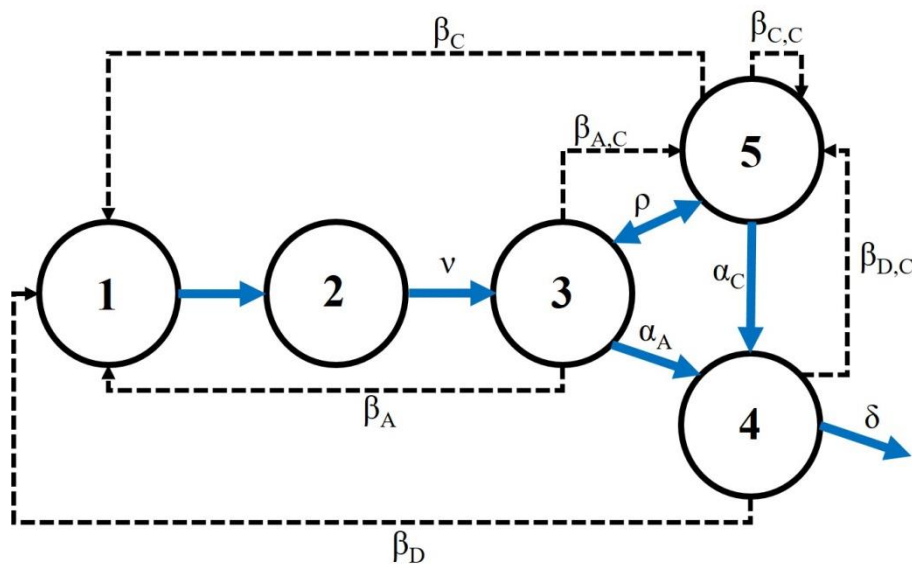


Figure 1. Life cycle graph of the YHV complex in *shrimp P. monodon*. 1) susceptible shrimp, 2) shrimp carrying YHV before becoming apparent signs, 3) shrimp with acute YHV infection, 4) dead shrimp, 5) shrimp with chronic YHV infection. β_D) Transmission coefficient from dead infected - susceptible shrimp, β_A) transmission coefficient from acute infection - susceptible shrimp, β_C) transmission coefficient from chronic infection - susceptible shrimp, $\beta_{D,C}$) transmission coefficient from dead infected carcass - chronic shrimp, $\beta_{A,C}$) transmission coefficient from acute infection - chronic shrimp, $\beta_{C,C}$) transmission coefficient from chronic infection - chronic shrimp, v) the infection coefficient, ρ) recovery coefficient to an acute to a chronic infection, α_A) mortality rate of an acute infection, α_C) mortality rate of a chronic infection and δ) decay coefficient of dead infected shrimp. Redrawn from Lotz, et al., 2005.

Yellow Head Virus-complex Life Cycle

Experimentally, YHV complexes have been transmitted by *per os* with infected carcasses, dip in contaminated water (Walker et al., 2001), direct injection for research issues and cohabitation with infected organisms (Soowanayan, 2015). Lotz et al. (2005) proposed a hypothetical life cycle for the YHV complex (Figure 1), where the chronic stage is more prominent (4, β_C , $\beta_{D,C}$, $\beta_{A,C}$, $\beta_{C,C}$ and α_C); in

asymptomatic chronic infections, (4) turns back to acute infections (3 and ρ) upon re-exposure, inducing increased complexity. This is a peculiarity of the YHV complex, where the kind of infected hosts that might serve as the source of exposures that would introduce a reversion remains unknown, and none of the parameters in this life cycle of YHV have been estimated. Cowley et al. (2002) reported that the YHV complex occurs by vertical transmission in the wild and in hatcheries, and this transmission develops from mother to offspring because the virions bind to the surface of the egg. Additionally, viruses were detected in the complex (e.g., GAV) in the spermatophore from infected male *P. monodon*, suggesting contamination by the male. Therefore, it has been shown that the complex YHV transmits vertically through contamination of the surface of the fertilized egg.

Transmission routes and host range

Pond water (transport and intake water) that carries YHV is the primary abiotic vector with fast dissemination and transmission to susceptible shrimp and by cannibalism of infected or moribund shrimp. Transmission of the YHV complex can occur by bringing infected but apparently healthy host crustaceans into offspring ponds and its introduction to a naive system through the use of different sources of infection, such as nets, feeding trays and other pond equipment.

Although the YHV complex can remain viable for up to 72 hours in salt water, it has been documented that YHV1 of these complexes can be inactivated by heating (60 °C/15 min) and exposing it to different solutions (chlorine 30 ppm, NaOH 2%/10 min, calcium hypochlorite 30 ppm, iodine compounds 250 ppm/30 min and formalin 3%/10 min). There are no reports of YHV-complex survival using other chemical compounds, but it is likely that these viruses are sensitive to oxidizing agents, SDS, nonionic detergents and lipid solvents (Stentiford et al., 2009).

Previous studies of experimentally infected *P. monodon* with YHV exposed frequent co-infections with secondary opportunistic pathogens such as bacteria and other viruses such as Hepatopancreatic Parvovirus (HPV), WSSV (Wang and Chang, 2000) and Monodon Baculovirus (MBV), playing an important role in mortalities caused by YHV and not for opportunistic pathogens (Stentiford et al., 2009). In these studies, on YHV, as for other shrimp viral pathogens, the infectious doses documented are tissue-purified volumes due to the lack of available crustacean cell lines.

Within YHV, the main hosts are *P. monodon* black tiger shrimp from Asia; however, infections have occurred in *Penaens japonicus*. On the other hand, being YHV a closely related virus to GAV, the susceptibility of a species to this last one is very related to which presents YHV in the same species (Spann et al., 2000) likewise *Farfantepenaens merguensis* and *Penaens esculentus* are susceptible to the infection and the development of the GAV disease and it is reported that *F. merguensis* from Thailand has been experimentally infected with YHV and has been maintained as a carrier of latent infection in culture systems (Flegel, 2006). In the same way, *P. vannamei* and *P. stylirostris* are not known as natural hosts, but they are susceptible to infection by injection or feeding with YHV-infected tissue (Lu et al., 1994).

In *P. vannamei*, there are reports that show the presence of shrimp with signs of the disease, such as loss of body color and mortalities of 60-70%, in farms of the central part of Thailand, where it is believed that the origin of these outbreaks comes from a natural reservoir, and in this species, economic losses of up to 3 million dollars have been reported between 2007 and 2008 (Senapin et al., 2010). YHD has caused economic losses estimated at 500 million dollars since its discovery until 2006 (Ligthner, 2007). In other palaemonids, such as *Palaemonetes pugio*, under some conditions, this serves as a reservoir of the virus, while blue crab serves only as a carrier (Ma et al., 2010). In addition, *Metapenaeus affinis* was more susceptible to intramuscular injection and feeding than *Metapenaeus brevicornis*; nonetheless, both species of shrimp are considered carriers that can survive for long periods of time after infection, unlike some species of crabs, in which intramuscular infection by feeding does not proliferate, contrary to what happens with WSSV in these very organisms (Longyant et al., 2006). Currently, the red claw crayfish *Cherax quadricarinatus* is reported as the ideal carrier for YHV because it is susceptible, highly tolerant and transmits this virus without showing any signs of YHD (Soowannayan et al., 2015).

Nomenclature and Taxonomy of YHV

Initially, YHV was classified as a *Baculovirus* by Limsuwan in 1991, and it had a DNA core since its cytoplasmic nature, development, size and morphology were suggested. In addition to being known as a granular yellow head baculovirus, until that moment, it was not a new virus in Thailand but one that had been recently accepted by a dramatic increase in occurrence and severity, with the possible causes being an increase in reservoirs of the virus due to an increase in the activity or intensity of culture farms. Later, Wongteerasupaya et al., 1995 mention that YHV is an RNA virus, similar to *Rhabdovirus* or *Coronavirus*, which was provisionally classified as a *Rhabdovirus*. Currently, YHV is classified inside the order of *Nidovirals* that are mostly recognized for infecting mammals (*Coronavirus*, *Torovirus* and *Arterivirus*) but also some infect birds (*Coronavirus*) or invertebrates (*Ronivirus*).

Nidoviruses cause a large variety of diseases, and they can vary from asymptomatic, persistent and carrier to cause fatal infections (Pasternak et al., 2006). Officially since 1996, the genera *Coronavirus*, *Torovirus* and *Arteriviridae* have joined the family *Coronaviridae*, which was initially considered unrelated to YHV, and recently, the family *Roniviridae* was admitted within the domain of invertebrate hosts.

The taxonomy acquires its name of the singular strategy of Nidovirus to express all genes located downward of the replicase gene of a nested cluster of sgRNA of 3' coterminial (from latin nidus=nest). However, the most important reason for this unification was found in the replicase gene itself, and phylogenetic analysis of the replicase domain, including the RNA dependence of the RNA polymerase (RdRP) and the helicase, grouped different Nidoviruses, indicating that all of them have a common ancestor (Cowley et al., 2000; Pasternak et al., 2006).

YHV is a member of the family *Roniviridae*, and the name of this family is generated in reference to the form of virions that are shown as rod-shaped and gender *Okavirus* comes of the preference that

this YHV presents in the lymphoid organ named OKA because of its principal affection tissue (Mayo, 2002).

Genotypes of the YHV complex

YHV and GAV viruses are highly complementary in their genomes, although they present considerable differences that have helped to classify them as geographic topotypes or genetic lines (Wijegoonawardane et al., 2008). Recently, it was reported that at least eight different geographic topotypes of YHV were obtained by analyzing sequences of the ORF1b gene. These genetic lines are distributed in most of *P. monodon*'s natural geographic range in Africa, Asia and Australia. Among the assigned geographic topotypes, genotype 1 (YHV1) includes the reference chain of YHV (GenBank AY052786), which is the only agent capable of causing YHD. Several YHV-causing partial sequences in shrimp have been reported in GenBank, and some of them show tiny variations in nucleotides; however, the Thai sequence (THA-00D-11) and three Taiwan viruses (TWN-00-D1, TWN-00-D2 and TWN-00-D3) are equal.

Genotype 2 includes the reference chain of GAV (AF227196), the Australian virus of healthy or affected shrimp with mid-crop mortality syndrome or MCMS (AUS-97-MCMS3, AUS-97-MCMS1 and AUS-97-MCMS2), some Vietnamese viruses of healthy post larvae (VNM-02-H6, VNM-02-H64, VNM-01-H65 and VNM-01-H77) and Thai viruses (THA-03-HA, THA-03-HB, THA-03-HG and THA-04-HK). The third filogenetic group (genotype 3) comprises all healthy breeder viruses from Thailand and Sarawak and other viruses found in healthy post larvae from Vietnam (subgroup formed by VNM-01-H41, VNM-01-H42, and VNM-02-H70).

Genotype 4 includes detected viruses in three batches of healthy post larvae in Nellore India (IND-02-H5, IND-02-H9, and IND-02-H7). Genotype 5 comprises viruses derived from juvenile shrimps from Malaysia (MYS-03-H4) and Thailand (THA-03-SG21) that showed slower growth, a batch of healthy post larvae sampled in the Philippines (PHL-03-H8) and a virus found in *P. vannamei* in Thailand (YHV ORF 1b gene); the relation between these four viruses from genotype 5 was more dispersed than viruses grouped inside the other lineages (Wijegoonawardane et al., 2008). Genotype 6 is tightly related to genotype 2 (GAV) and comprises viruses found from Mozambique (MOZ-04-H6, MOZ-04-H8, MOZ-04-H9, MOZ-04-H11 and MOZ-04-H12).

In 2015, genotype 7 (YHV7) was reported, and it was identified in *P. monodon* breeders with high mortalities collected in 2012 in Queensland Australia ponds, which came initially from the José Bonaparte Gulf. However, the role of the new YHV7 genotype in this episode of the disease is still unknown (Mohr et al., 2015). There are significant differences between the different genotypes of the YHV complex. YHV7 is a virulent pathogen similar to YHV1 that can cause culture mortality a few days after the appearance of the first signs of the disease (Callinan and Jiang, 2003). Genotype 8 (YHV8) was isolated from China in July 2012 and is related to YHV1, both of which form a monophyletic group.

The complete genome of YHV8 is 26,769 nucleotides with three open reading frames (ORFs) (Dong et al., 2017). YHV1 has 26,662 nucleotides distributed in four ORFs, while GAV is smaller and has a 26,235 nucleotide genome due to significant deletions in its intergenic regions, but 79% of its nucleotide sequence is identical to that of YHV1 (Cowley and Walker, 2002; Sittidilokratna et al., 2008). Nevertheless, GAV has an additional ORF that can be expressed in slight infections on shrimp tissues (Cowley *et al.*, 2004, Cowley and Walker, 2008). The analysis of sequences of genotypes 3, 4 and 5 indicates that their genome organization and transcription strategy are more related to GAV than to YHV1 (Wijegoonawardane et al., 2008). The complete genome of YHV8 was described in co-infection with AHPND (Dong et al., 2017), to which no genotype has been assigned. Recently, Li et al. (2018) established the purification and isolation of intact viral particles of genotype 8 of YHV isolated from China that had not been purified, where the author mentions that the rate of centrifugation for obtaining viral particles is decisive for obtaining full virions.

Detection Methods

To diagnose YHD, as in all diseases in aquaculture, certain basic steps and methods must be considered to detect YHV (etiological agent). These include anamnesis, clinical examination (clinical signs), microscopy (optical and electronic), bacteriology, histology, tests based on antibodies, molecular methods, immunological parameters and bioassays, depending on the pathology (Cuéllar-Anjel, 2008).

Specifically, the history of YHD, as a presumptive diagnosis, has been reported as an excessive consumption of food between 50-70 days of juvenile *P. monodon* culture, preceded by an abrupt cessation of feeding and subsequent mortality of 100%. Between these periods, a yellowish coloration of the hepatopancreas and cephalothorax can be observed, both with soft texture (Lightner et al., 2012). Among the microscopic analyses for YHD that are performed is the hemolymph smear, as a preliminary/presumptive diagnosis of this disease. A smear of hemolymph YHD positive reveals hemocytes with pycnotic and karyorrhexic nuclei without the presence of opportunistic bacteria. Another presumptive diagnosis is the histological analysis of shrimp gill tissue showing severe multifocal to diffuse necrosis with denso-basophilic spherical cytoplasmic inclusions (Flegel, 2006), while acute and chronic shrimp (A and C, respectively, Figure 1) add stomach necrosis (Lightner, et al., 1999).

To reinforce the aforementioned presumptive analysis, confirmatory dot blot or ELISAs are performed simultaneously using monoclonal antibodies against the gp116 coat glycoprotein of YHV (scFv). However, the limit of detection for the dot-blot assay is 9 ng of YHV, while with ELISA, it is 45 ng; therefore, the dot-blot/scFv test turned out to be more sensitive, simple and fast to use in the field (Intorasoot et al., 2007).

To date, the Manual of Diagnostic Tests for Aquatic Animals of OIE (2019) suggests 2 molecular methodologies for the detection of YHD: reverse transcription PCR (RT-PCR) and *in situ* hybridization (ISH). Among the RT-PCR analyses, 3 different protocols have been proposed, where the first detects

only the YHV1 genotype without detecting the other genotypes (Wongteerasupaya et al., 1995). Multiple nested RT-PCR is the second protocol that distinguishes YHV1 from YHV2 or GAV (Cowley et al., 2004) and identifies the YHV8 genotype in both stages of the test (Liu et al., 2014) while only detecting the genotype YHV7 in the first stage of RT-PCR (Mohr et al., 2015).

The last RT-PCR protocol is also a nested and multiple tests, which detects the YHV1-YHV7 genotypes without differentiating them (Wijegoonawardane et al., 2008b). For this, the sequence analysis of each RT-PCR product must be performed. It should be mentioned that this protocol, unlike the second, is incapable of detecting the YHV8 genotype. In addition, Khawsak et al. (2008) reported that the primers used in multiple nested RT-PCR protocols do not cross with other viruses, such as WSSV, TSV and IHNV. Although several ISH methods have been reported for YHD, the OIE proposes the protocol described by Tang et al. (2002), with which YHV1 and YHV2 (GAV) can be identified. The OIE recommends that for these molecular methods, fresh tissues of gills, lymphoid organs and hemolymph should be used.

Currently, a real-time PCR (qPCR) test for YHV is not available in the OIE's manual. Soowannayan et al. (2013) documented the effect of the antiviral tunicamycin against YHV in *P. monodon*, where they used an RT-qPCR assay to determine YHV copy numbers in hemolymph and hemocytes, without emphasizing the importance of this assay to detect this virus. In contrast, Arseneau and Laflamme (2016) implemented a methodology based on RT-qPCR with a detection limit of 170 copies of YHV, referencing analytical details of their assay. Similarly, in 2016, Yang and his team of collaborators designed an isothermal PCR-based system for the detection of at least two YHV complex genotypes, genotype 8 and genotype 1, with which they performed pathogen detection by detecting 7×10^7 , which was compared to conventional real-time PCR. Thus, Cowley et al. (2019) reported a real-time nested PCR method to identify YHV genotype 7 using ORF1b as a region of the rest of the genotypes that can be identified until 10 copies of the genome are identified mainly in pleopods and gills, with the two methods for the detection of YHV being efficient.

Viral blocking of YHV as a strategy

The viral blocking theory suggests that organisms previously infected with any viral pathogen acquire partial protection against a YHV infection, according to reports by Aranguren et al., (2012) who reported a previous infection with TSV to avoid a YHV infection, which suggests that viral interference exists between TSV-YHV, which could explain the absence of YHD in America, where TSV is present in culture ponds very often. RNA interference (RNAi) is a cell mechanism that is triggered by a double stranded RNA (dsRNA) fragmented by a Dicer enzyme generating small interfering RNA (siRNA), which is incorporated into the complex RISC (RNA-induced silencing complex) targeting the complementary mRNA fragmenting it into siRNA and inhibiting their translation.

The RNAi pathway is well documented, proving its effectiveness in abolishing the viral infections of pathogenic viruses in humans, such as poliovirus (Gitlin et al., 2002), human immunodeficiency virus HIV-1 (Novina et al., 2002), hepatitis C (Randall et al., 2003), and hepatitis B (Gilad et al., 2003). This suggests that this mechanism can be used as a tool for the prevention of viral diseases in aquaculture.

Maningas et al. (2008) was the first group to report the silencing of the transcription and translation of clotting protein (CP) and transglutaminase (TGase), components of the humoral response in the shrimp innate immune system. Inoculation of dsRNA homologous to TGases and CP inhibited the coagulation of hemolymph in vivo, and susceptibility to infection with *Vibrio penaeicida* and WSSV increased in dsRNA-treated organisms. Currently, treatment with RNAi is used to determine the function of at least two antimicrobial peptides (AMPs) in shrimp, the antilipopolysaccharide factor (ALF) and crustin. ALF silencing in *P. vannamei* challenged with *V. penaeicida* and *Fusarium oxysporum* significantly increased the susceptibility of shrimp to these pathogens (De la Vega et al., 2008). This shows the function of these genes in the immune activity of the shrimp.

Shochey et al. (2008) demonstrated the antimicrobial activity of crustins (AMPs) in *P. vannamei* challenged with *Vibrio penaeicida* previously injected with dsRNA of crustin isoforms. In this study, they suppressed crustin expression and later increased mortality in crustin-depleted shrimp infected with *V. penaeicida*. Amparyup et al. (2009) observed that gene silencing of prophenoloxidase (proPO) in *P. monodon* was more susceptible to *Vibrio harveyi*, while Fagutao et al. (2009) obtained an increase in the mortality of *Marsupenaeus japonicus* shrimp without bacterial or viral challenge when silencing proPO, registering an increase in the bacterial load in the hemolymph. Both studies concluded that the proPO system is an important component in the immune defense of the shrimp.

Therefore, RNAi technology provides an efficient tool to analyze other genes in shrimp, such as the β -integrin gene that inhibits WSSV infection (Li et al., 2007), as well as to explain the function of other genes involved in the molting, growth and reproduction of shrimp, such as gonad-inhibiting hormone (Treeratrakool et al., 2008), among others involved in the antiviral response. Reports of the function of dsRNA in shrimp show that the exposure of *P. vannamei* to dsRNA induces antiviral innate immunity against WSSV and TSV (Mudagandur et al., 2009). Lower replications of YHV were observed in cells of primary culture of shrimp that were transfected with dsRNA directed to nonstructural genes of the virus (Tirasophon et al., 2005), and the inhibition of YHV by dsRNA resulted in significantly lower mortalities in *P. monodon* (Yodmuang et al., 2006; Tirasophon et al., 2007). As a prophylactic strategy, Srisapoome et al (2018), reported the use of a byproduct of paper pulp, known as Lignine Kraft, to treat a YHV infection in shrimp, thus stating that the concentration of 200 mg/L lignin Kraft does not affect the health of organisms; however, it does not work as a preventative. By direct injection, there is no difference between the concentration of hemocytes, but if it favors phagocytosis, lignin Kraft pre-incubated for two hours with YHV and then delivered via promotes mortality rates by 50-60% at 14 days

of exposure. They conclude that the use of lignin byproducts for the treatment of YHV in shrimp should be reviewed in more detail.

CONCLUSIONS

Once the main features of this pathogen are known, it is easy to deduce that it is a highly fickle enemy, with an impressive capability of change that greatly limits the existing mitigation strategies. In Mexico, the attack of viral pathogens mainly in the northeast has left considerable sequels, so the presence of a character of this nature would have serious effects on production systems. Therefore, it is considered crucial to develop strategies that allow us to better understand the conditions of propagation and their effects on the environmental conditions of Mexican shrimp culture.

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Editors



Dr. Leandris Argente Martínez

Profesor e Investigador Titular “C” del Tecnológico Nacional de México, Campus Valle del Yaqui (ITVY). Miembro del Sistema Nacional de Investigadores, Nivel 1 (**SNI-1**). Profesor Perfil Deseable (**PRODEP**) de la Secretaría de Educación Pública de México, **Líder del Cuerpo Académico ITVAYA-CA-3**. Líneas de investigación: Fisiología Vegetal, Bioquímica, Biología Celular y Molecular en plantas y microorganismos. Doctorado en Ciencias Biotecnológicas. Desarrollo de investigaciones sobre mecanismos fisiológicos, rutas anapleróticas y mecanismos moleculares activados por los organismos durante su adaptación a condiciones adversas como el cambio climático. Aplicación de técnicas experimentales univariadas y multivariadas para el monitoreo de germoplasmas a través de indicadores moleculares. Uso de marcadores

moleculares de tolerancia de los organismos al estrés abiótico (salinidad, sequía y calor). Manejo de técnicas de isótopos estables para el seguimiento de reacciones bioquímicas en células y tejidos. Síntesis de metabolitos secundarios en plantas con fines farmacológicos. Entre sus principales proyectos, se encuentra vigente en 2022 “Aplicaciones del microbioma y el metaboloma de la *Parkinsonia aculeata* L. Sp. Pl. para la mitigación de estreses biótico y abiótico en el semidesierto y en especies de interés agrícola en México” correo electrónico para contacto: oleinismora@gmail.com.



Dra. Ofelda Peñuelas Rubio

Profesor e Investigador Titular “C” del Tecnológico Nacional de México, Campus Valle del Yaqui (ITVY). Miembro del Sistema Nacional de Investigadores, Nivel 1 (**SNI-1**). Profesora con Perfil Deseable (**PRODEP**) de la Secretaría de Educación Pública de México, miembro del Cuerpo Académico ITVAYA-CA-3. México. Realizó dos estancias posdoctorales (Enero 2016-Diciembre 2017) dentro del programa de Estancias Nacionales de CONACYT en el Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional unidad Sinaloa del Instituto Politécnico Nacional en el área de Ecología Molecular de la Rizósfera. Es Doctora en Ciencias especialidad en Biotecnología. Su quehacer científico lo desarrolla en el área agrícola, principalmente en el manejo sustentable de los recursos implicados en los agroecosistemas y el

aprovechamiento de la microbiota del suelo. Ha participado en colaboración con distintos grupos de investigación lo que le ha permitido participar en proyectos multidisciplinarios y en publicaciones científicas. Email para contacto: ofeperub@gmail.com.



Pantanal Editora
Rua Abaete, 83, Sala B, Centro. CEP: 78690-000
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